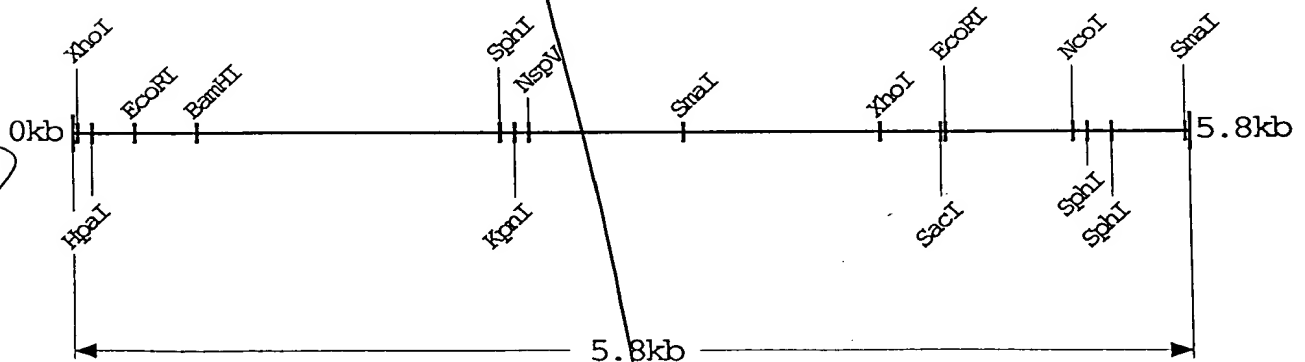


WHAT IS CLAIMED IS:

1. A DNA fragment of about 5.8 Kb containing a toluene monooxygenase gene, having 1 BamHI restriction site, 2 EcoRI restriction sites, 1 HpaI restriction site, 1 KpnI restriction site, 1 NcoI restriction site, 1 NspV restriction site, 1 SacI restriction site, 2 SmaI restriction sites, 3 SphI restriction sites, 2 XhoI restriction sites, no ClaI restriction site, no DraI restriction site, no EcoRV restriction site, no HindIII restriction site, no NdeI restriction site, no NheI restriction site, no PvuII restriction site, no ScaI restriction site, no Sse8387I restriction site, no StuI restriction site, and no XbaI restriction site, and having a restriction map of:



2. The DNA fragment according to claim 1, wherein the DNA fragment has a nucleotide sequence of SEQ ID NO: 1 in the Sequence Listing.

3. A DNA fragment having a nucleotide sequence of

SEQ ID NO: 1 with deletion, substitution, and/or addition of one or more nucleotides encoding a protein having a toluene monooxygenase activity.

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4. A recombinant DNA comprising a vector enabling maintenance or replication in a host and a DNA fragment according to any one of claims 1 to 3.

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5. The recombinant DNA fragment according to claim 4, wherein the vector can be maintained or replicate in a bacterium.

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6. A DNA fragment containing a region encoding a toluene monooxygenase, the region comprising a first sequence encoding a polypeptide TomL having an amino acid sequence of SEQ ID NO: 3, a second sequence encoding a polypeptide TomM having an amino acid sequence of SEQ ID NO: 4, a third sequence encoding a polypeptide TomN having an amino acid sequence of SEQ ID NO: 5, a fourth sequence encoding a polypeptide TomO having an amino acid sequence of SEQ ID NO: 6, and a fifth sequence encoding a polypeptide TomP having an amino acid sequence of SEQ ID NO: 7 of the Sequence Listing, and the first to fifth sequences are aligned so that expressed TomL - TomP polypeptides can form an active monooxygenase protein.

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7. The DNA fragment according to claim 6, wherein no spacer sequence is present between the first to fifth sequences or at least one spacer sequence is present between the first to fifth sequences.

8. The DNA fragment according to claim 6 or 7, further comprising a sequence encoding a polypeptide TomQ having an amino acid sequence of SEQ ID NO: 8 in the Sequence Listing.

9. A DNA fragment containing a region encoding a toluene monooxygenase, wherein the region comprises a first sequence encoding a polypeptide TomL having an amino acid sequence of SEQ ID NO: 3, a second sequence encoding a polypeptide TomM having an amino acid sequence of SEQ ID NO: 4, a third sequence encoding a polypeptide TomN having an amino acid sequence of SEQ ID NO: 5, a fourth sequence encoding a polypeptide TomO having an amino acid sequence of SEQ ID NO: 6, and a fifth sequence encoding a polypeptide TomP having an amino acid sequence of SEQ ID NO: 7, and the first to fifth sequences are aligned so that expressed TomL - TomP polypeptides can form an active monooxygenase protein;

wherein in at least one of the first to fifth sequences of the DNA fragment, deletion, substitution, and/or addition of one or more nucleotides are present

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in the proviso that the toluene monooxygenase protein is active.

5 10. A DNA fragment comprising a region encoding a polypeptide TomK the polypeptide TomK having an amino acid sequence of SEQ ID NO: 2, and a property to enhance the toluene monooxygenase activity of a protein comprised of at least TomL to TomP; or a region encoding a variant TomK in which the amino acid
10 sequence of SEQ ID NO: 2 is altered with the proviso that the property to enhance the toluene monooxygenase activity is not impaired.

15 11. A recombinant DNA comprising a vector, a promoter, and the DNA fragment according to any one of claims ^{6, 7, or 9} ~~6 to 9~~, and the vector and the promoter are functionally ligated to the DNA fragment to enable expression of the toluene monooxygenase encoded by the DNA fragment.

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12. The recombinant DNA according to claim 11 wherein the promoter and the vector can function in a bacterium.

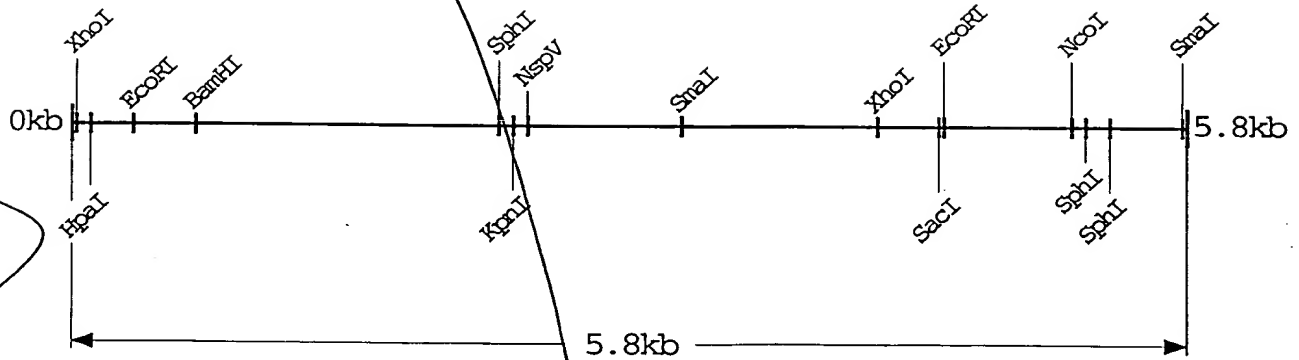
25 13. A recombinant DNA comprising a vector; a first promoter and the DNA fragment encoding polypeptide TomK according to claim 10 wherein the DNA

fragment for TomK polypeptide is functionally linked to the first promoter to be expressed by the first promoter; a second promoter and the DNA fragment according to any one of claims 6 to 9 wherein the DNA fragment is functionally linked to the second promoter to be expressed by the second promoter.

14. The recombinant DNA according to claim 13, wherein the first and second promoters and the vector can function in a bacterium.

15. A transformant obtained by introducing a recombinant DNA into a host microorganism, the recombinant DNA comprising a vector enabling maintenance or replication in a host and a DNA fragment of about 5.8 Kb containing a toluene monooxygenase gene having 1 BamHI restriction site, 2 EcoRI restriction sites, 1 HpaI restriction site, 1 KpnI restriction site, 1 NcoI restriction site, 1 NspV restriction site, 1 SacI restriction site, 2 SmaI restriction sites, 3 SphI restriction sites, 2 XhoI restriction sites, no ClaI restriction site, no DraI restriction site, no EcoRV restriction site, no HindIII restriction site, no NdeI restriction site, no NheI restriction site, no PvuII restriction site, no ScaI restriction site, no Sse8387I restriction site, no StuI restriction site, and no XbaI restriction site, and having a restriction

map of:



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16. The transformant according to claim 15,
wherein the host microorganism is a bacterium.

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17. A transformant obtained by introducing a
recombinant DNA into a host microorganism, where the
recombinant DNA comprises a vector enabling maintenance
or replication in a host, and a DNA fragment ligated
thereto having a nucleotide sequence of SEQ ID NO: 1 of
the Sequence Listing with deletion, substitution
and/or addition of one or more nucleotides, still
encoding an active toluene monooxygenase.

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18. The transformant according to claim 17,
wherein the host microorganism is a bacterium.

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19. A transformant obtained by introducing a
recombinant DNA comprising a vector, a promoter and a
DNA fragment into a host microorganism where the DNA

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fragment contains a region encoding a toluene monooxygenase, the region comprising a first sequence encoding a polypeptide TomL having an amino acid sequence of SEQ ID NO: 3, a second sequence encoding a polypeptide TomM having an amino acid sequence of SEQ ID NO: 4, a third sequence encoding a polypeptide TomN having an amino acid sequence of SEQ ID NO: 5, a fourth sequence encoding a polypeptide TomO having an amino acid sequence of SEQ ID NO: 6, and a fifth sequence encoding a polypeptide TomP having an amino acid sequence of SEQ ID NO: 7, and the first to fifth sequences are aligned so that ^{expressed} ~~toluene~~ TomL - TomP polypeptides can form an active monooxygenase protein;

wherein the promoter and the DNA fragment are functionally linked enabling expression of the toluene monooxygenase protein encoded by the DNA fragment.

20. The transformant according to claim 19, wherein said host microorganism is a bacterium.


~~21. A method for producing a toluene monooxygenase, comprising a step of making the transformant according to any one of claims 15, 17 and 19 produce a toluene monooxygenase that is a gene product of the recombinant DNA introduced into the transformant.~~

22. A method for degrading at least one of a chlorinated aliphatic hydrocarbon compound and an aromatic compound in a medium comprising a step of degrading at least one of a chlorinated aliphatic hydrocarbon compound and an aromatic compound by using the transformant according to any one of claims 15, 17 and 19.

23. The degradation method according to claim 22, wherein the medium is an aqueous medium.

24. The degradation method according to claim 22, wherein the medium is soil.

25. The degradation method according to claim 22, wherein the medium is air.

26. The degradation method according to claim 22, wherein the chlorinated aliphatic hydrocarbon compound is either trichloroethylene (TCE) or dichloroethylene (DCE).

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25 ~~27.~~ The degradation method according to claim 22, wherein the aromatic compound is at least one of toluene, benzene, phenol, and cresol.

28. A method for cleaning a medium polluted with

at least one of a chlorinated aliphatic hydrocarbon compound and aromatic compound comprising a step of degrading at least one of a chlorinated aliphatic hydrocarbon compound and an aromatic compound using the transformant according to any one of claims 15, 17 and 19.

10 29. The cleaning method according to claim 28 wherein the medium is an aqueous medium.

30. The cleaning method according to claim 28 wherein the medium is soil.

15 31. The cleaning method according to claim 28 wherein the medium is air.

20 32. The cleaning method according to claim 28 wherein the chlorinated aliphatic hydrocarbon compound is either trichloroethylene (TCE) or dichloroethylene (DCE).

33. The cleaning method according to claim 28 wherein the aromatic compound is at least one of toluene, benzene, phenol, and cresol.

34. A method for remedying an environment polluted with a pollutant being at least either of a

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chlorinated aliphatic hydrocarbon compound or an aromatic compound, comprising a step of degrading the pollutant by using the transformant according to any one of claims 15, 17 and 19.

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35. The remediation method according to claim 34 wherein the environment is made of an aqueous medium.

10 36. The remediation method according to claim 35 wherein the polluted aqueous medium is brought into contact with a carrier holding the transformant.

15 37. The remediation method according to claim 36 wherein the contact is carried out by placing the carrier holding the transformant in a container, introducing the polluted aqueous medium from one side of the container, and discharging the remedied aqueous medium from another side.

20 38. The remediation method according to claim 34, wherein the environment is made of soil.

25 39. The remediation method according to claim 38 being carried out by introducing an aqueous medium containing the transformant into the polluted soil and supplying nutrients and/or oxygen for proliferation of the transformant in the polluted soil.

40. The remediation method according to claim 39 wherein the transformant is introduced in the soil with applying pressure through an injection well provided in the polluted soil.

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41. The remediation method according to claim 38 wherein the polluted soil is introduced in a liquid phase containing the transformant.

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42. The remediation method according to claim 38 wherein the polluted soil is brought into contact with a carrier holding the transformant.

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43. The remediation method according to claim 34 wherein the environment is made of air.

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44. The remediation method according to claim 43 wherein the polluted air is introduced into a liquid phase containing the transformant.

45. The remediation method according to claim 43 wherein the polluted air is brought into contact with a carrier holding the transformant.

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46. The remediation method according to claim 45 wherein contact is carried out by placing the carrier holding the transformant in a container, introducing

polluted air from one side of the container, and
discharging cleaned air from another side.

47. The remediation method according to claim 34
5 wherein the chlorinated aliphatic hydrocarbon compound
is either trichloroethylene (TCE) or dichloroethylene
(DCE).

48. The remediation method according to claim 34
wherein the aromatic compound is at least one of
toluene, benzene, phenol, and cresol.

49. A component polypeptide having any one of
amino acid sequences of SEQ ID NOs: 2 to 8 in the
15 sequence listing, capable of being a component of a
toluene monooxygenase.

50. A toluene monooxygenase comprising at least
component polypeptides TomL to TomP having amino acid
20 sequences of SEQ ID NOs: 3 to 7 in the Sequence
Listing.

51. The toluene monooxygenase according to claim
50 further comprising a component polypeptide TomQ
25 having an amino acid sequence of SEQ ID NO: 8 in the
Sequence Listing.

52. The toluene monooxygenase according to claim 50 further comprising a component polypeptide TomQ having an amino acid sequence of SEQ ID NO: 8 in the Sequence Listing.

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53. The toluene monooxygenase according to claim 52 further comprising a component polypeptide TomQ having an amino acid sequence of SEQ ID NO: 8 in the Sequence Listing.

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54. A variant toluene monooxygenase comprising at least component polypeptides TomL-TomP of amino acid sequences of SEQ ID Nos. 3 to 7 wherein one or more amino acids have been deleted from, substituted to, and/or added to the polypeptides with the proviso that the enzyme activity is not impaired.

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55. A recombinant DNA comprising a vector, a promoter, a first DNA fragment being the DNA fragment of any one of claims 6 to 9, and a second DNA fragment being the tomK DNA fragment of claim 10, wherein the first DNA fragment is functionally connected to the promoter to express an active toluene monooxygenase, and the second DNA fragment is functionally connected to the promoter to express a property to enhance the toluene monooxygenase activity.

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